RESEARCH NOTES

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Seroprevalence of *Toxoplasma gondii* Antibodies in Wild Dolphins From the Spanish Mediterranean Coast

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ABSTRACT: Although *Toxoplasma gondii* infection has been found occasionally in cetaceans, little is known of the prevalence of antibodies to *T. gondii* in wild dolphins. Antibodies to *T. gondii* were determined in serum samples from 58 dolphins stranded in the Spanish Mediterranean coast. Modified agglutination test was used to determine *T. gondii* antibodies, and a titer of 1:25 was considered indicative of *T. gondii* infection. Antibodies to *T. gondii* were found in 4 of 36 striped dolphins (*Stenella coeruleoalba*), in 2 of 4 common dolphins (*Delphinus delphis*), in 4 of 7 bottlenose dolphins (*Tursiops truncatus*), and in 1 harbour porpoise (*Phocoena phocoena*). Antibodies were not found in 9 Risso's dolphins (*Grampus griseus*) and in 1 long-finned pilot whale (*Globicephala melas*) surveyed. The results indicate that *T. gondii* infection is frequent in at least 3 dolphin species from the Mediterranean Sea.

Toxoplasma gondii infections have been reported in many species of warm-blooded animals, including several species of marine mammals (Dubey and Beattie, 1988; Resendes et al., 2002; Dubey et al., 2003). Recently, concerns have been raised that *T. gondii* is a cause of mortality in southern sea otters (*Enhydra lutris nereis*), which are an endangered species in U.S. waters (Cole et al., 2000; Miller et al., 2002; Krueder et al., 2003). It has been suggested that sea otters become infected with *T. gondii* oocysts in the sea from freshwater coastal runoff (Miller et al., 2002). Although *T. gondii* infections have been found occasionally in Atlantic bottlenose dolphins (*Tursiops truncatus*) (Cruickshank et al., 1990; Inskeep et al., 1990; Di Guardo, Agrimi et al., 1995; Di Guardo, Corradi et al., 1995; Shulman et al., 1997; Jardine and Dubey, 2002), striped dolphin (*Stenella coeruleoalba*) (Domingo et al., 1992; Di Guardo, Agrimi et al., 1995; Di Guardo, Corradi et al., 1995), spinner dolphin (*S. longirostris*) (Migaki et al., 1990), Risso's dolphin (*Grampus griseus*) (Di Guardo, Agrimi et al., 1995; Resendes et al., 2002), and in a Tucuxi (*Sotalia fluviatilis*) (Bandoli and Oliveira, 1977), little is known of the prevalence of antibodies to *T. gondii* in wild dolphins. The present study reports the seroprevalence of *T. gondii* antibodies in the 4 most abundant wild species of Mediterranean dolphins: striped dolphin (*S. coeruleoalba*), bottlenose dolphin (*T. truncatus*), common dolphin (*Delphinus delphis*), and Risso's dolphin (*G. griseus*). Moreover, it also includes findings in 1 harbour porpoise (*Phocoena phocoena*) and 1 long-finned pilot whale (*Globicephala melas*).

All animals in this study were fresh dead or live-stranded on the Spanish Mediterranean coast. In necropsied dolphins, whole blood was collected from the heart, and in live-stranded animals, samples were collected from the ventral fluke vein. All samples were collected in commercial, sterile serum collector tubes and centrifuged. Sera were then frozen at −80 C until tested. Data on species and sex were recorded for each animal. Samples were provided by different groups from cetacean stranding network: Institut Cavanilles (University of Valencia) from Comunitat Valenciana, Aula del Mar from Andalucia, and Centre de Recuperació d'Animals Marins de Catalunya (CRAMC) from Catalonia. The major part of the territorial area of the Spanish Mediterranean coast was covered by these 3 working groups. Sera were analyzed for *T. gondii* antibodies with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987), with minor modifications. Briefly, sera were filtrated, using a sterile 0.2-μm microfilter (Nalgene®, Rochester, New York), to eliminate erythrocytes from hemolysis and any bacterial membranes present in the samples and diluted at 1:25, 1:50, and 1:500 dilutions. Hemolysis of sera is not a major problem for detection of *T. gondii* antibodies with MAT using a 1:25 dilution of serum and using the mouse-derived tachyzoites (Dubey et al., 2003). Filtration showed no interference with titration of the positive control serum used. Positive and negative controls were included in each test. Sera with doubtful results were reexamined.

Although the specificity and sensitivity of MAT have not been evaluated for the diagnosis of toxoplasmosis in marine mammals, it is the most evaluated and specific test for the diagnosis of toxoplasmosis in animals, particularly pigs (Dubey et al., 1995; Dubey, 1997). A titer of ≥1:25 was considered indicative of *T. gondii* infection in dolphins as has been for other species (Dubey and Beattie, 1988; Dubey et al., 2003). The prevalence of infection was statistically analyzed considering the variables species, habitat, and sex. It was not possible to determine species-specific age although length measurements of each animal were performed.

Data were analyzed using the SPSS 11.0 Statistical Program by chi-square test. The differences between variables were analyzed by Bonferroni or Tukey–Kramer tests. For multiple comparison, Dunn's test was used, and when variances were not homogenous, a nonparametric test was performed. The differences were considered statistically significant when $P \leq 0.05$. When the number of studied individuals in a species was less than 3, that species was not included in the statistical analysis.

Antibodies (MAT ≥ 1:25) to *T. gondii* were found in 4 of 7 *T. truncatus*, with titers of 1:25 in 2 and 1:50 in 2; in 2 of 4 *D. delphis*, with titer of 1:25 in 1 and 1:500 in the other; in 4 of 36 *S. coeruleoalba*, with titer of 1:25 in 3 and 1:500 in 1; and in 1 *P. phocoena*, with titer of 1:25. Antibodies to *T. gondii* were not found in sera of 9 *G. griseus* and the *G. melas*.

Species with coastal habits in the study area (*T. truncatus* and *D. delphis*) had significantly higher prevalence of *T. gondii* antibodies (6 of 11 animals, 54.4%) than pelagic species (*S. coeruleoalba* and *G. griseus*, 4 of 45 animals, 8.8%) ($P = 0.003$). No significant difference was observed in the prevalence of infection and sex. Antibodies were found in 13.0% of 23 male dolphins and in 22.8% of 35 female dolphins. The postmortem studies in 14 of 58 dolphins excluded active toxoplasmosis.

This is the first report on seroprevalence of *T. gondii* infection in several cetacean species from the Mediterranean Sea. A high seroprevalence was found in *T. truncatus* and *D. delphis*, but antibodies were also detected in *S. coeruleoalba* and *P. phocoena* species. To our knowledge, prevalence of *T. gondii* antibodies has only been investigated in *D. leucas* and *T. truncatus* (Mikaelian et al., 2000; Dubey et al., 2003).

Clinical toxoplasmosis has been described in wide range of dolphin species (Bandoli and Oliveira, 1977; Cruickshank et al., 1990; Inskeep et al., 1990; Migaki et al., 1990; Domingo et al., 1992; Di Guardo, Agrimi et al., 1995; Di Guardo, Corridi et al., 1995; Shulman et al., 1997; Mikaelian et al., 2000; Jardine and Dubey, 2002; Resendes et al., 2002). Most of these cases were associated with an immunosuppressive state caused by a morbillivirus infection. *Toxoplasma gondii* has been thought to be a sporadic infection in dolphins although the seroprevalence results from this study and from the one by Dubey et al. (2003) indicate that this infection is rather frequent at least in some species.

Although no seropositive *G. griseus* was detected in our study, clinical infection has been found in 2 Mediterranean animals of this species.

The fact that higher *T. gondii* titers were found in coastal rather than in pellagic dolphins suggests that their habitat can influence the probability of infection. Marine animals could become infected by oocysts washed into the sea via coastal freshwater runoff contaminated with cat excrement. This hypothesis has been strongly supported by studies of Miller et al. (2002) in sea otters. *Toxoplasma gondii* oocysts can survive in seawater for at least 6 mo (Lindsay et al., 2003).

Toxoplasma gondii infection in dolphins is intriguing because they drink little water and feed on fish, squid, or other cold-blooded sea animals. Dolphins could become infected by the ingestion of water contaminated with oocysts, aquatic birds, or infected meat thrown into the sea. The role of marine invertebrates in the life cycle of *T. gondii* is unknown. *Toxoplasma gondii* is not known to parasitize any cold-blooded animal; however, molluscs can filter large quantities of water and may thus concentrate microbes from the water. Experimentally, *T. gondii* oocysts have been concentrated by molluscs and shown to remain infectious to mice (Lindsay et al., 2001; Arkush et al., 2003).

The present study indicated that *T. gondii* infection is frequent, even if it does not necessarily cause disease in western Mediterranean dolphins. Furthermore, at least 4 different Mediterranean species of dolphins can be infected, with the parasite being particularly prevalent in the coastal dolphin species.

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